



**INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE
EGAS MONIZ**

MESTRADO EM NUTRIÇÃO CLÍNICA

**EFFECT OF THE INGESTION OF A MOUSSE WITH CINNAMON
C. BURMANNII ON THE POSTPRANDIAL BLOOD GLUCOSE
RESPONSE OF HEALTHY SUBJECTS AND ITS ANTIOXIDANT
POWER**

Trabalho submetido por
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para a obtenção do grau de Mestre em Nutrição Clínica

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Abstract

Background: Cinnamon has been shown to reduce postprandial glycaemia and enhance insulin sensitivity in healthy adults.

Aims: To study the effect of *C. burmannii* on the postprandial blood glucose response of healthy subjects and its antioxidant capacity in a semi-solid food.

Design: Twenty four apparently healthy subjects participated in this study. They were randomly assigned in group A (reference meal) or group B (test meal). The blood glucose concentrations were measured before the ingestion of the meals and 30, 60, 90 and 120 minutes after the start of the meal. The test meal used consisted of 100 g of mousse mixed with 3 g of cinnamon.

Results: The addition of 3 g of cinnamon to the mousse had no significant effect in blood glucose response in terms of the areas under the curve (AUC) and in the different postprandial times ($p>0,05$). The mean Cmax was significantly lower after the ingestion of the reference meal than after the ingestion of the mousse with 3 g of *C.burmannii* (96 mg/dl VS 104,42 mg/dl; $p=0,011$). The chemical analysis showed that the mousse with 3 g of cinnamon has a much higher phenolic content and antioxidant capacity than the mousse without cinnamon.

Conclusions: The inclusion of cinnamon in the mousse increased the antioxidant capacity of this semi-solid food, however it did not reduce the postprandial glucose response in healthy subjects.

Key-words: *Cinnamomum burmannii*, antioxidant capacity, mousse, postprandial blood glucose.

Resumo

Enquadramento: Em indivíduos saudáveis, tem sido demonstrado que a canela reduz a glicémia pós-prandial e melhora a sensibilidade à insulina.

Objectivos: Estudar o efeito da *C. burmannii* na resposta glicémica pós-prandial de indivíduos saudáveis e a sua capacidade antioxidante num alimento semi-sólido.

Metodologia: Vinte e quatro indivíduos, aparentemente saudáveis, participaram neste estudo. Foram aleatoriamente distribuídos no grupo A (refeição padrão) ou no grupo B (refeição teste). As concentrações de glicose no sangue foram medidas antes da ingestão das refeições e 30, 60, 90 e 120 minutos após o início da refeição. A refeição teste consistiu em 100 g de mousse com 3 g de canela.

Resultados: A adição de 3 g de canela à mousse não teve um efeito significativo na resposta glicémica em termos da área abaixo da curva (AUC) e nos diferentes tempos pós-prandiais ($p>0,05$). O valor médio de Cmax foi significativamente mais baixo após a ingestão da refeição padrão, do que após a ingestão da mousse com 3 g de *C.burmannii* (96 mg/dl VS 104,42 mg/dl; $p=0,011$). A análise química revelou que a mousse com 3 g de canela possui um teor em fenóis e uma capacidade antioxidante muito maior do que a mousse sem canela.

Conclusões: A inclusão de canela na mousse aumentou a capacidade antioxidante deste alimento semi-sólido, no entanto, não reduziu a glicémia pós-prandial de indivíduos saudáveis.

Palavras-Chave: *Cinnamomum burmannii*, capacidade antioxidante, mousse, níveis de glucose pós-prandiais.

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List of Abbreviations

AUC - Area under the curve

BMI - Body mass index

C_{max} - Maximum concentration

CP – Cinnamon polyphenols

FRAP - Ferric reducing antioxidant potential

GE – Gastric emptying

GI – Glycemic index

GIP - Glucose-dependent insulintropic polypeptide

GLP-1 - Glucagon-like peptide-1

GLUT4 - Glucose transporter 4

IFG - Impaired fasting glucose

IR β - Insulin receptor β

PTA - Phosphotungstic acid

R24 - Dietary recall

SEM - Standard error of the mean

SMM – Skeletal muscle mass

TAS - Total antioxidant capacity

TEAC - Trolox equivalent antioxidant capacity

TTP - Tristetrapolin

T2DM - Type 2 Diabetes Mellitus

WHR – Waist to hip ratio

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1. Introduction

It is known that numerous spices have medicinal properties and beneficial effects on health (Shan, Cai, Sun & Corke, 2005). Cinnamon leaves and bark are used extensively as spices (Vasanthi & Parameswari, 2010). Cinnamon from Sri Lanka is the cinnamon commonly commercialized and comes from the tree *C. verum*, which belongs to the family Lauraceae. *C. Burmannii* is native from Southeast Asian and Indonesia, and is cultivated with commercial purposes in the Islands of Indonesia (Hardman, 2004).

Besides being used as a spice, cinnamon has also been used as a medicinal herb. It was mainly used for gastrointestinal complaints but nowadays cinnamon has been associated with a positive effect on postprandial glucose metabolism, being the treatment of type 2 diabetes mellitus (T2DM) the most promising field of research for cinnamon (Ulbricht *et al*, 2011). Besides this association with glucose and insulin metabolism, it is described that cinnamon has various potential effects such as anti-inflammatory, antibacterial, antifungal, and antioxidant properties (Shan *et al*, 2005; Shan *et al*, 2007; Ulbricht *et al*, 2011) and these effects are associated with their constituents. The nutritional and chemical composition of cassia bark has been studied, and range as it follows: moisture (6.5-11.9%), crude fiber (12.0-28.8%), carbohydrate (6.9-32.0%), protein (3.1-3.4%), fixed oil (0-2.1%), volatile oil (0.5- 5.1%). Among these, the constituent of commercial importance is the volatile oil (Sangal, 2011). Shan (2007) indicated that (E)-cinnamaldehyde was the predominant volatile oil component in cinnamon stick and the crude extract of cinnamon stick also contained high levels of nonvolatile compounds, mainly proanthocyanidins and (epi)catechins. Although cinnamaldehyde and eugenol are the major compounds, the chemical composition of cinnamon varies considerably depending on the specie, the age of the plant, the segment sampling, the size, length and weight of the bark, and the extraction method (Woehrlin, Fry, Abraham & Preiss-Weigert, 2010). The diversity of constituents of several *C. burmannii* extracts are represented in **Table 1**.

Table 1 – Extract composition of Cinnamon Stick (*C.burmannii*)

Reference	Extract	Compounds
Anderson <i>et al</i> , 2004	Aqueous	Procyanidin Type-A oligomers; chlorogenic acid, ferulic acid, t-cinnamic acid, guaiacol, cinnamic acid methyl ester, homovanillic acid, cinnamide, isovanillic acid cinnam, 2-methoxy-cinnamaldehyde, cinnamyl alcohol, 3-methoxy-l-tyrosine, clove oil, 4-oxo-4h-1-benzopyran-carboxylic acid, p-coumaric acid, resveratrol, o-coumaric acid, vanillic acid, curcumin, vanillin azine, eugenol.
Thantsin <i>et al</i> , 2008	Oily	Camphor (1.79%), 4-terpineol (0.50%), cinnamaldehyde (2.70%), δ -elemene (2.32%), α -cubebene (1.56%), α -ilangene (0.52%), caryophyllene (1.23%), epi-bicyclosesquiphellandrene (0.44%), calarene (0.94%), β -guaiene (2.14%), aromadendrene (1.47%), α -humulene (0.43%), santalene (0.77%), α -amorphene (5.39%), valencene (0.60%), α -muurolene (2.40%), γ -cadineno (0.50%), δ -cadinene (5.98%), patchoulene (0.25%), α -calacorene (1.71%), cyclopentadecane (1.29%), caryophyllenyl alcohol (0.49%), γ -eudesmol (1.28%), T-cadinol (1.15%), α -eudesmol (1.63%), pentadecanol (0.96%), isocurcumenol (0.55%), palmitic acid (2.48%) elaidinsaeure (7.71%).
He <i>et al</i> , 2005	Methanolic	Cinnamaldehyde (0.038%), cinnamyl alcohol (0.02%), eugenol.
Shan <i>et al</i> , 2007	Methanolic	Procyanidin B1 (0,7 %) and B2 (5,12%), procyanidin trimer (13,76%), (+)-catechin (2,61%), procyanidin dimer (1,27%), procyanidin tetramer (2,35%), (-)-epicatechin (1,02%), (E)-cinnamic acid (2,69%), (E)-cinnamaldehyde (62,18%), (S)-cinnamaldehyde (1,95%).
Cao <i>et al</i> , 2010	Aqueous	Procyanidin Type-A polymers, galocatechin galate, coumarin, epicatechine gallate, cinnamic acid.

The compounds present in *C.burmannii* that have an antioxidant effect and also seem to exhibit insulin-like activity in cells, have been identified as procyanidin type-A (doubly linked) polymers (Anderson *et al*, 2004; Cao, Polansky & Anderson, 2007; Cao, Graves & Anderson, 2010). Anderson (2004) found out that these polyphenolic compounds, present as monomers or oligomers, are responsible for insulin enhancing activity in epididymal fat cells, *in vitro*.

To explore the molecular basis of insulin-like activity of cinnamon with doubly-linked procyanidin type-A polymers, studies (Cao *et al*, 2007; Cao *et al*, 2010) were developed to investigate the effects of cinnamon polyphenols (CP) on the regulation of insulin receptor β (IR β), glucose transporter 4 (GLUT4) and, anti-inflammatory protein, tristetrapolin (TTP) in mouse 3T3-L1 adipocytes. These three proteins are involved in the insulin signaling transduction pathway. Their results suggest that the mechanism of cinnamon's insulin-like activity may be in part due to increases in the amounts of proteins involved in insulin signaling, such as TTP, IR β , and GLUT4. Based on these results and previous studies (Jarvill-Taylor, Karalee, Anderson & Graves, 2001; Anderson *et al*, 2004), Cao (2010) proposed a model (see **Figure 1**) which shows that cinnamon polyphenols may interfere in multiple steps of the insulin signaling transduction pathway:

- (1) activating insulin receptors by increasing their tyrosine phosphorylation activity and by decreasing phosphatase activity that inactivates the receptor (Imparl-Radosevich *et al*, 1998);
- (2) increasing glycogen synthase activity and glycogen accumulation (Jarvill-Taylor *et al*, 2001);
- (3) decreasing GSK3 β activity, which phosphorylates TTP, leading to an increase in TTP activity (Jarvill-Taylor *et al*, 2001);
- (4) increasing the amount of TTP, IR β , and GLUT4 proteins.

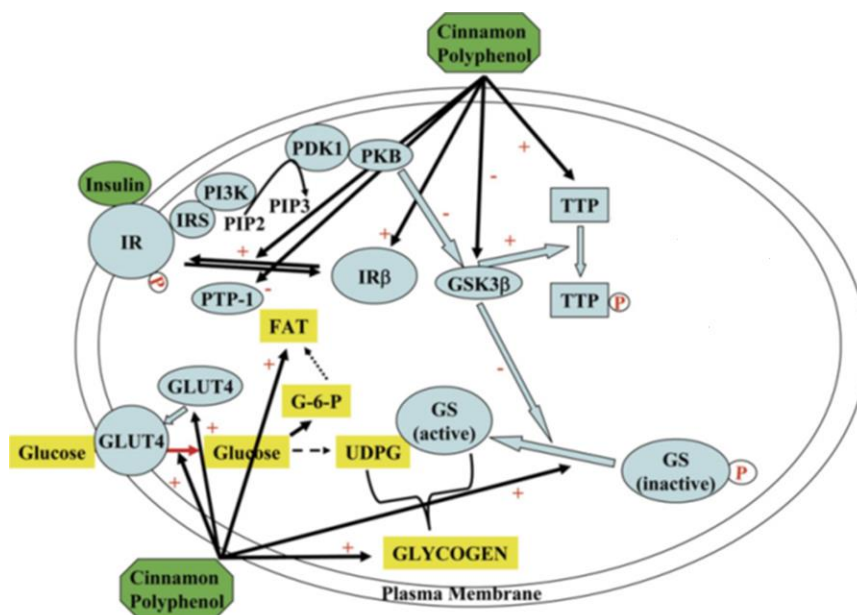


Figure 1 - A model of actions by CP in the insulin signal transduction pathway. Adapted from Cao (2007)

By increasing the amount of IR β protein, GLUT4 and TTP in the cells (Cao *et al*, 2010), these polyphenols improve insulin sensitivity and glucose uptake in adipocytes, leading to more efficient glucose transport and utilization (Anderson, 2008; Ulbricht *et al*, 2011). In addition, CP-induced TTP accumulation in 3T3-L1 adipocytes may have beneficial effects on the condition of diabetic people by down-regulating the synthesis of pro-inflammatory cytokines (Cao *et al*, 2007). These phenolic compounds are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzymes (Shan *et al*, 2005).

Taking into account these potential mechanisms of cinnamon polyphenols *in vitro*, several clinical trials have been developed to evaluate the effects of cinnamon in humans. Some of those trials are summarized in **Table 2**:

Table 2 - Clinical trials with cinnamon

Reference	Intervention	Sample (n/type)	Time	Significant effects
Khan <i>et al</i> , 2003	<i>Cinnamomum cassia</i> (1, 3, and 6 g/day)/sulfonylurea drugs	60/T2DM	40 days	Significant decreases in fasting glucose (11.6 VS 9,7 mmol/l with 1g/day; 11,4 VS 9,9 mmol/l with 3g/day; 13,0 VS 11,4 mmol/l with 6g/day), cholesterol, triglycerides and low-density lipoprotein.
Safdar <i>et al</i> , 2004	<i>Cinnamomum cassia</i> (1, 3, and 6 g/day)	60/T2DM	60 days	Significant reduction in the mean fasting glucose levels after 60 days (208,7 VS 174,6 mg/dl with 1 g/day; 206,2 VS 177,8 mg/dl with 3 g/day; 233,9 VS 205,7 mg/dl with 6g/day)
Mang <i>et al</i> , 2006	3 g per day/oral antidiabetics	79/T2DM	4 months	Significantly reduction in fasting plasma glucose concentration (9,26 mmol/l VS 8,15 mmol/l)
Ziegenfuss <i>et al</i> , 2006	500 mg of dried aqueous extract of cinnamon per day	22/Pre-diabetic and metabolic syndrome	12 weeks	Significant decreases in fasting blood glucose levels (116,3 VS 106,5 mg/dl), increases in lean mass and decreases in body fat.
Hlebowicz <i>et al</i> , 2007	<i>Cinnamomum cassia</i> (6 g with test meal)	14/healthy normal BMI subjects	Simultaneously with food (1 time)	Significantly reduced postprandial glucose (at 15, 30 and 45 min) and decreased gastric-emptying rate.
Solomon and Blannin, 2007	<i>Cinnamomum cassia</i> (5 g with glucose)	7/healthy normal BMI male subjects	12 hours	No significant decreases in total plasma glucose response; improved oral glucose tolerance and insulin sensitivity.
Crawford, 2009	<i>Cinnamomum cassia</i> (1 g per day)/oral antidiabetics	109/T2DM	90 days	Lowered HbA1C 0.83%
Hlebowicz <i>et al</i> , 2009	<i>Cinnamomum cassia</i> (1 and 3 g with test meal)	15/healthy normal BMI subjects	Simultaneously with food (1 time)	3g reduced postprandial insulin and increased GLP-1. No significant effects were seen in postprandial blood glucose.
Roussel <i>et al</i> , 2009	250 mg of dried aqueous extract of cinnamon, twice a day	22/Overweight or obese with impaired fasting glucose	12 weeks	Increase of FRAP and plasma SH groups, decreased plasma MDA levels; Significantly reduced fasting glucose levels (114 VS 102 mg/dl).
Soni, 2009	<i>Cinnamomum cassia</i> (2 g, 4 times a day)	30/T2DM	40 days	Significantly reduced fasting and postprandial blood glucose levels (148 VS 120 mg/dl and 187 VS 163 mg/dl, respectively).
Markey <i>et al</i> , 2011	<i>Cinnamomum zeylanicum</i> (3 g with the test meal)	9/healthy normal BMI subjects	Simultaneously with food (1 time)	No evidence was found for changes in gastric emptying parameters, postprandial glucose concentrations, oxidative stress, arterial function or appetite.
Magistrelli and Chezem, 2012	<i>Cinnamomum cassia</i> (6 g with test meal)	15 normal BMI and 15 obese subjects	Simultaneously with food (1 time)	No significant difference in blood glucose between the two BMI groups at any time point. In a combined analysis of all subjects, the addition of cinnamon reduced 120-minute glucose AUC and blood glucose at 15, 30, 45 and 60 minutes. At 120 minutes, blood glucose was significantly higher with cinnamon consumption (91 VS 109 mg/dl).

Considering the observation that cinnamon enhances insulin activity *in vitro*, Khan, Mahpara, Mohamma, Khan & Richard (2003) conducted the first randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of cinnamon in individuals on sulfonylurea drug therapy with type 2 diabetes. A total of 60 subjects were divided randomly into six groups. The first three groups were given 1, 3 or 6 g of *Cinnamomum cassia* in pill form daily, while the other three groups consumed the same amounts of placebo capsules. After 40 days, all participants in the groups taking cinnamon reduced fasting glucose, cholesterol, triglycerides and low-density lipoprotein, however no significant changes were observed in the group taking the placebo. The study indicated a benefit of cinnamon supplementation at low levels (1-6 g/day).

Several clinical trials on the effect of cinnamon in diabetic subjects have been conducted since then. Hlebowicz, Darwiche, Björgell & Almér (2007) conducted a study in healthy subjects, in which the addition of 6 g of cinnamon to rice pudding significantly delayed gastric emptying and lowered the postprandial blood glucose levels. Hlebowicz (2007) concluded that the reduction in the postprandial blood glucose response observed after the ingestion of cinnamon could be partially explained by the reduction in gastric emptying rate, since this is a major factor in blood glucose homeostasis in normal subjects.

However, not all clinical trials have reported a reduction on fasting and postprandial blood glucose levels after the consumption of cinnamon or extracts of cinnamon. Markey *et al* (2011) developed a single-blind, randomized, crossover study in nine healthy subjects. The subjects had to consume a test meal that consisted of three pancakes served with 20 g of chocolate spread and 300 ml of water, with 8 gelatin capsules, totaling 3 g of cinnamon, or a wheat flour placebo. Nevertheless, the supplementation of 3 g of cinnamon did not alter the postprandial response to a high-fat test meal and no changes in gastric emptying parameters, postprandial triacylglycerol, glucose concentrations, oxidative stress, arterial function or appetite were observed (Markey *et al*, 2011).

Although several clinical trials have been developed, there are few studies in healthy subjects in which the ingestion of cinnamon, in powder, occurs simultaneously

with food. Taking this remark into account and given the dependence of the observed effects in regards to cinnamon species, quantities, types of consumed foods and sample, this study aims to assess if the intake of a mousse with cinnamon versus the intake of a mousse without cinnamon has a different impact on postprandial glycaemia of healthy subjects, and characterize the antioxidant capacity of the two meals.

2. Objectives

The purpose of this investigation is to study the effect of cinnamon *C. burmannii* on the postprandial blood glucose response of healthy subjects and characterize the antioxidant capacity of a semi-solid food with and without 3g of *C. burmannii*.

2.1. Specific Objectives

- Ascertain the antioxidant capacity of the mousse with and without cinnamon *C. burmannii*;
- Determine, in healthy subjects, if there are significant differences in the mean values of capillary blood glucose at different postprandial times, after the ingestion of a mousse without cinnamon and after the ingestion of a mousse with 3 g of *C.burmannii*;
- Determine, in healthy subjects, if there are significant differences in the areas under the curve for postprandial blood glucose response of healthy subjects, after the ingestion of a mousse with and without 3 g of *C.burmannii*.

3. Methodology

3.1. Subjects

The subjects were recruited from the student population of Instituto Superior de Ciências da Saúde Egas Moniz, were all over 18 years old and healthy.

3.2. Study design

The study was designed by adaptation of the method described by Hlebowicz (Hlebowicz *et al*, 2007), Figure 2, and approved by the Ethics Committee of Instituto Superior de Ciências da Saúde Egas Moniz (Annex A).

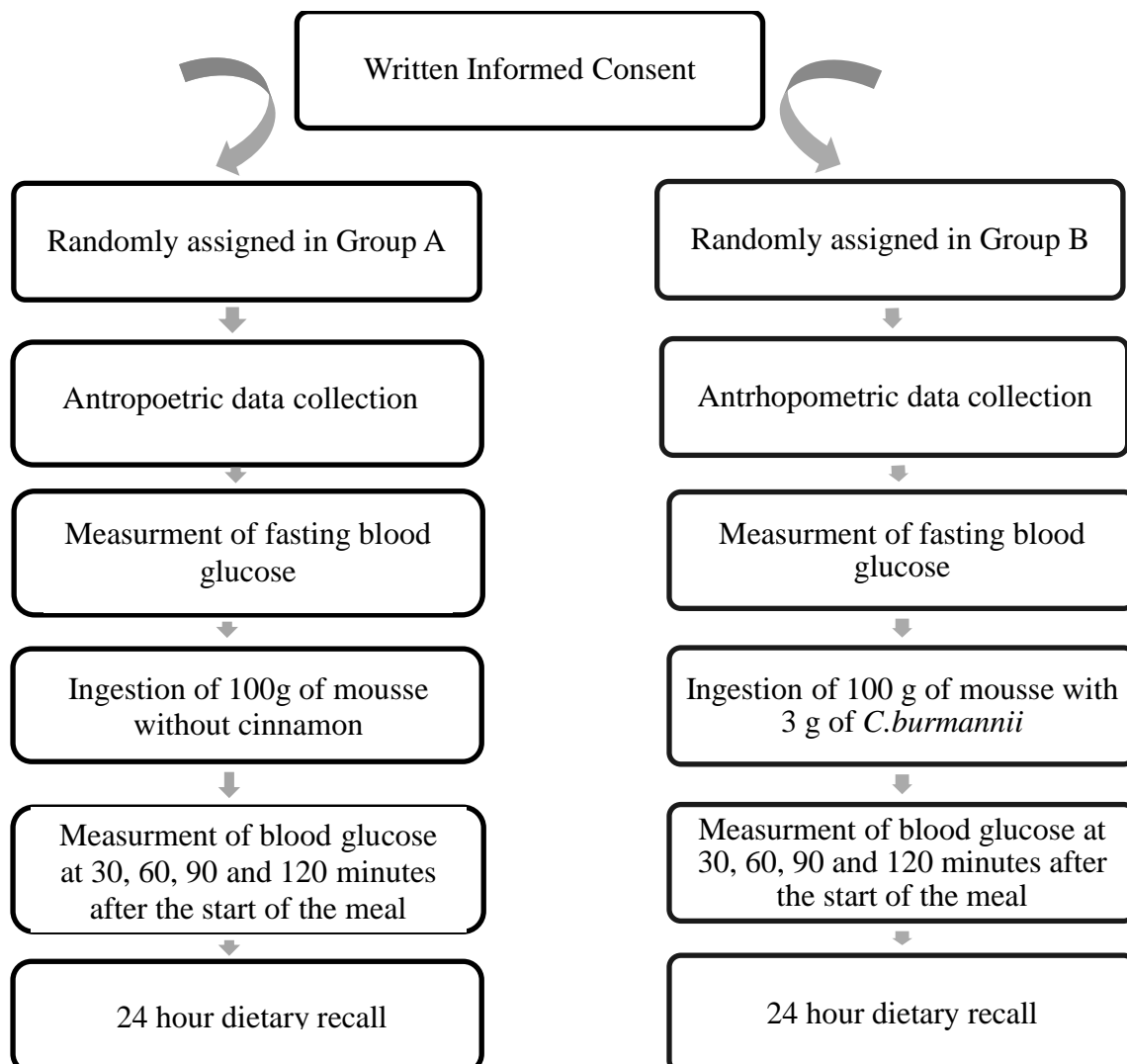


Figure 2 – Study design

All the subjects gave their written informed consent (Annex B) before the study began and knew that they could withdraw from the study at any time. They were randomly assigned in group A (reference meal) or group B (test meal) and were required to have a normal fasting blood glucose level on the day of the study. The subjects were examined in the morning between 8:00 and 12:00 after an 8 hour fasting. Finger-prick capillary blood samples were collected before the ingestion of the meals and 30, 60, 90 and 120 minutes after the start of the meal to determine the blood glucose levels. Blood glucose concentrations were measured with the OneTouch Vita® glucometer, which has an accuracy of 98%. The meals were ingested within 5 minutes.

The anthropometric data was recorded (Annex C) through bio-impedance body fat analyzer InBody® 230 and the height was measured by the stadiometer Craveira Jofre.

Each subject responded to a 24 hour dietary recall (R24, Annex D) to eliminate bias with regard to hours of fasting, composition of the meals and cooking process. No specific diet was imposed and the participants consumed their usual diet. The practice of physical activity was also evaluated through a questionnaire, and was also recorded in the clinical data of the subjects (Annex E).

3.3. Test meal preparation and constitution

The meal consisted of a mousse prepared by the investigator, with the following ingredients and amounts (for a total of 25 doses): 10 gelatin sheets, 1 l of skim milk, 12 egg yolks (204,1 g), 323 g of white sugar, 82,3 g of butter and 963,8 g of sour cream. The test meal consisted of 100 g of mousse mixed with 3 g cinnamon, which was milled with the coffee grinder Taurus Aromatic 150 W. Alimentary composition of the mousse was estimated through PIABAD® v.5.04 Software. The reference meal consisted of 100 g of mousse, without cinnamon.

3.4. Chemical Analysis

3.4.1. Chemicals and reagents

The reagents Ferric Chloride (III) hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), folin-ciocalteu (2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), TPTZ 2,4,6-tri(2-piridil)-s-triazine, ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), methanol (CH_3OH), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt and 1-buthanol ($\text{C}_4\text{H}_{10}\text{O}$) were purchased from Sigma-Aldrich, gallic acid-1-hydrate ($\text{C}_6\text{H}_2(\text{OH})_3\text{COOH} \cdot \text{H}_2\text{O}$) was purchased from Acros Organics and sodium carbonate (Na_2CO_3) was from ICS Science group.

The following solutions were prepared: chloridric acid 40 mM (HCl 37% purchased to Sigma-Aldrich), phosphate buffer pH=7 (NaH_2PO_4 e Na_2HPO_4 purchased to Scharlau), acetate buffer 300 mM pH=3,6 ($\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$ and CH_3COOH purchased to AnalaR Normapur).

3.4.2. Methodology

3.4.2.1. Extract preparation

The mousse was subjected to a hydro-ethanolic extract (20:80). Posteriorly, the mixture was filtered using the Whatman paper filter. Homogeneous samples were obtained and subjected to chemical analysis.

3.4.2.2. Total Phenolic Content

The total phenolic content was determined by adaptation of the method of Prabha and Vasantha (2011). The samples were analyzed in triplicate: 312,5 μl were pipette to an ethanol:water 80:20 (V/V) solution, to which was added 187,5 μl of water, 5 ml of solution Folin- Ciocalteu reagent (1:10 diluted with water) and 4 ml of aqueous solution Na_2CO_3 1M. A blank solution was prepared, in which the sample was replaced by a solution in ethanol:water 80:20 (V/V) and 187,5 μl of water. We waited for 15 minutes after agitation of the tubes and the absorbance was read at 765 nm.

Gallic acid was used as the standard solution ($Y=0,0034X+0,018$ ($R^2=0,9966$)) and the results were expressed in mg of gallic acid equivalents (GAE) / L.

3.4.2.3. Quantification of Proanthocyanidins

Quantification of proanthocyanidins was determined by an adaptation of the method described by Gu *et al* (2002). This method is based on the acid hydrolyze of proanthocyanidins polymers, to produce reddish chromophores such as cyanidin and delphinidin. The greater the absorbance value, the greater the content of proanthocyanidins.

All measurements were done in triplicate: 150 μ L of each sample was pipette, to capped tubes, to which was added 2850 μ l of HCl/1-buthanol solution (10% V/V). A blank solution was prepared, in which the sample was replaced by 150 μ l of ethanol:water (80:20). After agitation and incubation at 100 °C for 50 min, the absorbance was read at 550 nm using a Perkin Elmer Lambda 25 UV-Vis spectrophotometer.

3.4.2.4. Characterization of the antioxidant capacity of the meals

3.4.2.4.1. Ferric Reducing Antioxidant Potential (FRAP) assay

This method was adapted from the method described by Thaipong, Boonprako, Crosby, Cisneros-Zevallos & Byrne (2006) and is based on the capacity of the antioxidants to reduce Fe^{3+} , a colorless ferric complex to a blue-colored ferrous complex, Fe^{2+} in the presence of 2,4,6-tri(2-piridil)-s-triazine (TPTZ).

The FRAP solution was previously prepared, adding 25 ml of sodium acetate 300 mM pH 3.6, to 2,5 ml of 10 mM TPTZ in HCl 40mM, and 2,5ml of $FeCl_3 \cdot 6H_2O$ 20 mM. 150 μ l of the samples were added with 2850 μ l of FRAP solution. The mixture was kept in the dark for 30 minutes and then the absorbance was read at 593 nm. A blank solution was prepared, in which the sample was replaced by 150 μ l of water, in the same conditions.

The Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), vitamin E analogous, was used as standard ($Y=2,17 \times 10^{-3}X+2,32 \times 10^{-2}$ ($R^2=0,998$)) and results were expressed as $\mu\text{M TE}$.

3.4.2.4.2. Estimation of Total Antioxidant Capacity by the ABTS⁺

Method

The capacity of reduction of ABTS⁺ radicals by antioxidant compounds was studied through two similar tests: TAS (Total antioxidant capacity) and TEAC (Total equivalent antioxidant capacity), using the synthetic antioxidant Trolox as standard. The ABTS⁺ radical is formed by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) by enzymatic pathway (peroxidase TAS) and by the chemical reaction of potassium persulfate (TEAC).

The ABTS is a peroxidase substrate that when oxidized by peroxy radicals and other oxidants in the presence of hydrogen peroxide generates the meta stable radical cation ABTS⁺. The color of this cation is a dark-green, visible in a wavelength of 600 to 750 nm. The antioxidant power is determined by the ability to rescue this radical, resulting in a colorless product. As the antioxidant agent reacts with this cation, the color loses intensity, resulting in a decrease in absorbance values. Thus, the lower the absorbance value, the higher the concentration of antioxidant molecules (Karadag, Ozcelik & Saner, 2009).

3.4.2.4.3 TAS test

This test was executed in the equipment RANDOX, RX Daytona model, using the *Kit* RANDOX - NX 2332.

For each replicate of this analysis, 610 $\mu\text{mol/l}$ of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) were added to 6,1 $\mu\text{mol/l}$ of peroxidase (metmyoglobin) and 250 $\mu\text{mol/l}$ of hydrogen peroxide, in the presence and absence of the samples. The generation of the ABTS[•] radical was measured spectrophotometrically at 600 nm. The result was expressed as mM of trolox/l.

3.4.2.4.4 TEAC test

Previously a solution was prepared by adding 10 ml of ABTS• 7 mM to 176 µl of potassium persulfate 140 mM, which was incubated at room temperature in the dark for 12 hours. After this time, the solution was diluted in ethanol until it reached an absorbance of 0.7 at 734 nm (about 70 times) (Zulueta, Esteve & Frígola, 2009).

All measurements were done after pipetting 150 µl of the sample and 2850 µl of the ABTS solution in ethanol to capped tubes. A controlled solution was prepared, in which the sample was replaced by 150 µl of ethanol. The absorbance was read at 734 nm:

$$\%I = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

(Equation 1)

%I – percentage of inhibition

With the purpose of comparing the antioxidant power of the two meals, it was calculated the number of dilutions necessary for the inhibition percentage of the radical reached 50%.

3.5. Statistical Analysis

The database was organized on Microsoft® Excel 2010 software and the data analysis was carried out with the statistical analysis program SPSS v19.0. The areas under the curves (AUCs) of each subject were measured for blood glucose by using GRAPH PAD PRISM software (version 5.01). The AUC was calculated above zero and the AUC values are presented as means. The maximum concentration of postprandial blood glucose level observed, for each subject, is denoted as Cmax. Significant differences in the mean values of Cmax, fasting blood glucose, AUC levels and time of overnight fast between the two groups were evaluated with the use of a T-test for independent samples. The level of statistical significance was set at $p \leq 0,05$.

A Chi-Square test was applied to evaluate if there were significant differences in the prevalence of family history of diabetes between the two groups.

Energy, macronutrient and dietary fiber intake in the day prior to the intervention was estimated through The Food Processor v.10.5.0 software. A multiple linear regression was applied to evaluate if the alimentary composition of the last meal (in terms of total caloric value, carbohydrates, fat and protein) in the day before the intervention could influence the fasting glycemic values of each group.

Significant differences between the means of alimentary intake in the day prior to the intervention, in the two groups, were evaluated by a Mann-Whitney U test.

To determine whether there were differences in postprandial blood glucose mean levels at different times (30, 60, 90 and 120 min), between the two groups, a repeated-measures ANOVA and mixed model was applied.

To compare the body mass index (BMI), waist to hip ratio (WHR), skeletal muscle mass (SMM), fat mass and waist line means between the two groups, a multivariate analysis of variance (MANOVA) was applied. To model the relationship between the BMI, fat mass and waist line with the AUC of the two groups, a multiple linear regression was applied.

4. Results

4.1. Meals Characterization

The total caloric value of a portion (≈ 100 g) was 153 kcal: 14% of the energy being derived from protein (5,2 g), 45% from carbohydrate (17,3 g), and 41% from fat (7 g) (see **Table 3**):

Table 3 - Alimentary composition of the reference meal and test meal

<i>Amount</i>	<i>Reference Meal</i>	<i>Test meal (with 3g of cinnamon)</i>
Total energy (kcal)	153	160,5
Total protein (g)	5,2	5,3
Total Carbohydrate (g)	17,3	18,9
Mono and disaccharide sugars (g)	17,3	18,9
Total fat (g)	7	7,09

The alimentary composition of cinnamon was obtained in *Tabela da Composição de Alimentos* (Porto & Oliveira, 2006).

The results of the chemical analysis of the reference meal and the test meal (with cinnamon) are represented in **Table 4**:

Table 4 – Antioxidant characterization: Total Phenolic Content, Proanthocyanidins quantification, FRAP, TEAC and TAS tests of the meals with and without cinnamon *C.burmannii*

<i>Meal/Assay</i>	<i>Reference Meal</i>	<i>Test Meal (with 3 g of C. burmannii)</i>
Total Phenolic content (mg/l gallic acid)	326,8	1246,9
Proanthocyanidins (abs)	0,137	0,316
FRAP (μ mol TROLOX/l) (μ mol TROLOX/mg phenols)	91,1 0,279	941,4 0,755
TAS (mmol TROLOX/l)	<0,42	20,76
ABTS (IC 50 (n° of dilutions))	NA	11,8

NA- not available

With the addition of 3 g of *C.burmannii* to the mousse, the values of total phenolic content ranged from 326,8 mg/l to 1246,9 mg/l of gallic acid, a variation of more than 900 mg, providing this meal a higher phenolic content. The quantification of proanthocyanidins revealed that the test meal also has a higher amount of proanthocyanidins than the reference meal. In the FRAP assay, the values ranged from 91,1 to 941,4 $\mu\text{mol TROLOX/l}$ showing that the total antioxidant capacity of the mousse is ten times higher in the presence of cinnamon. The phenolic compounds present in cinnamon exhibit an antioxidant capacity, as observed by the range in the values of the FRAP assay, from 0,279 to 0,755 $\mu\text{mol TROLOX/mg phenols}$.

With the purpose of comparing the antioxidant power of the two meals, it was calculated the number of dilutions necessary for the inhibition percentage of the radical ABTS reached 50%. As observed in **Figure 3**, the mousse without cinnamon has no antioxidant capacity to reduce the ABTS radical at 50%. On the other hand, the mousse with cinnamon showed 50% inhibitory concentration achieved with 11,8 dilutions.

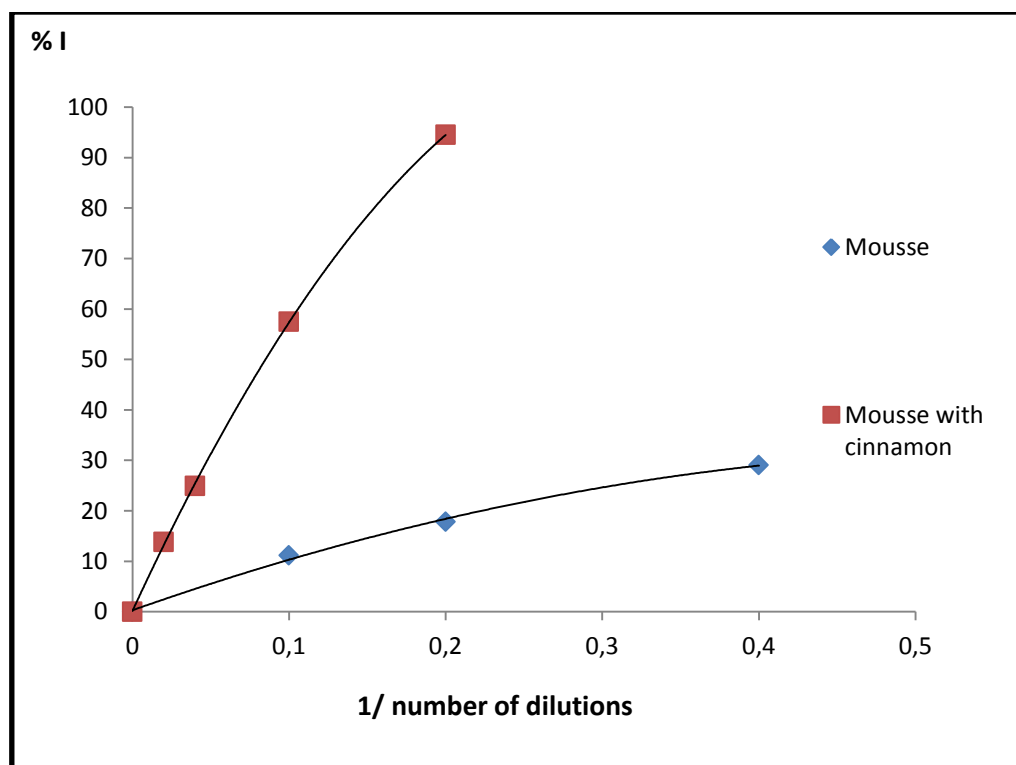


Figure 3 – Reduction of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation in the presence of *C.burmannii*.

4.2. Sample Characterization

Twenty four apparently healthy subjects participated in this study. Of these, 8 (33%) were males and 16 (67%) were females (**Figure 4**):

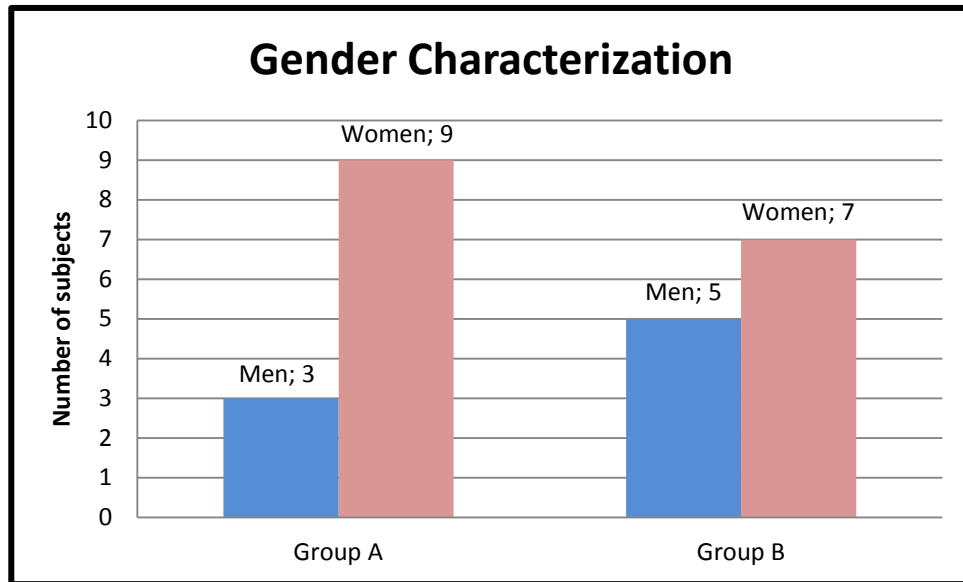


Figure 4 - Characterization of the sample by gender

Of the twenty four subjects, more than half were physically active ($n=15$), being this prevalence higher in the experimental group (Group B). On the other hand, the number of smokers ($n=6$) was also higher in group B (**Figure 5**):

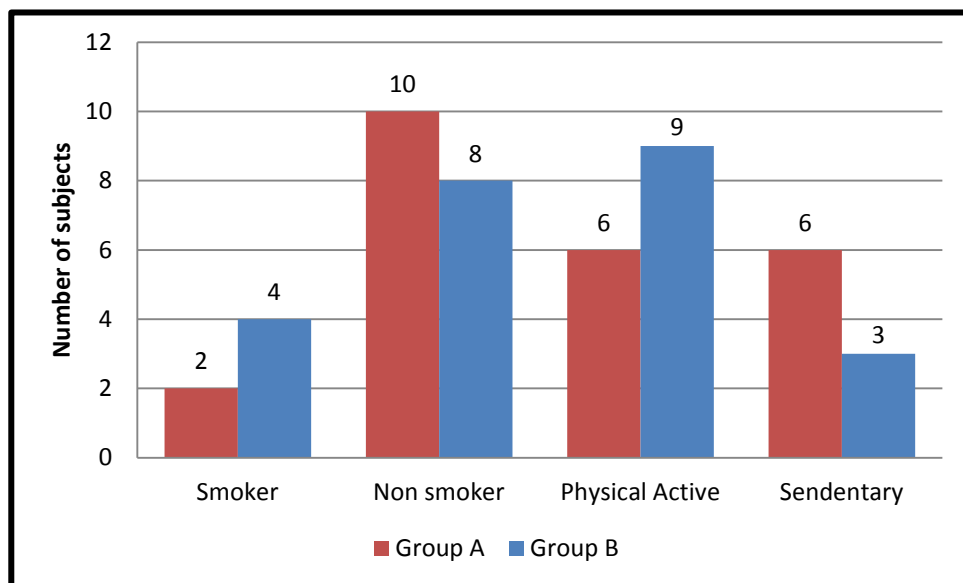


Figure 5 – Characterization of smoking habits and physical activity

Mean body mass index (BMI; in kg/m^2) was $22,4 \pm 2,3$ (range 17,9-26,2) in group A and $21,5 \pm 3,1$ (range 18,2-29,5) in group B. Of the twenty four subjects, none had a BMI higher than $29,9\text{kg/m}^2$. In **Figure 6**, is represented the sample distribution in terms of BMI, for the control group and experimental group.

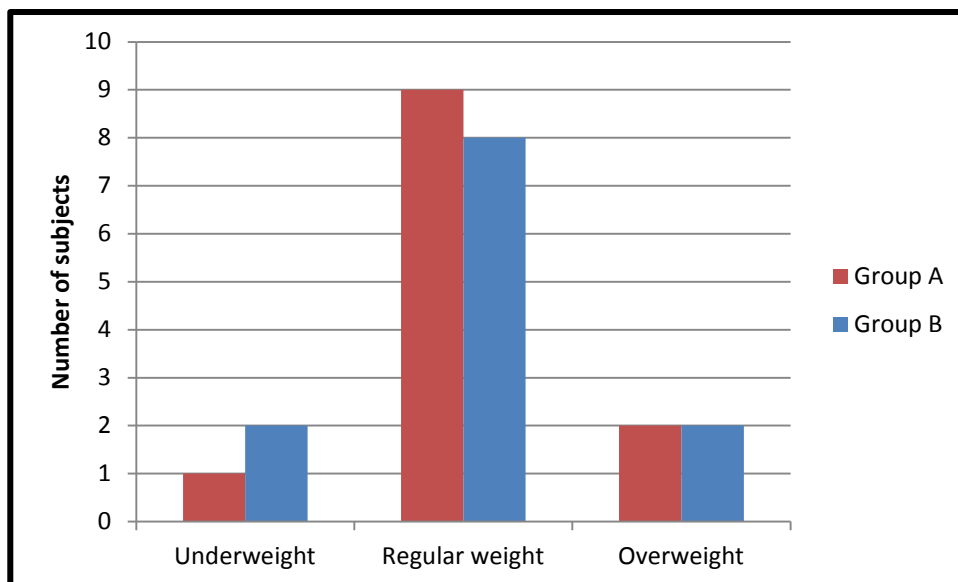


Figure 6 – Sample characterization by BMI classes (Underweight: $\text{BMI} < 18,5\text{kg/m}^2$; Regular weight: $18,5\text{ kg/m}^2 \leq \text{BMI} \leq 24,9\text{ kg/m}^2$; Overweight: $25\text{ kg/m}^2 \leq \text{BMI} \leq 29,9\text{ kg/m}^2$)

The anthropometric data, as well as mean age are represented in **Table 5**:

Table 5 – Age and anthropometric mean values of subjects in each group

	<i>Group A</i>	<i>Group B</i>
Age (years)	$25,1 \pm 10,8$	$25,5 \pm 3,3$
Weight (kg)	$59,9 \pm 7,6$	$60,7 \pm 11,6$
Height (cm)	$163,4 \pm 6,1$	$167,4 \pm 7,2$
BMI (kg/m^2)	$22,4 \pm 2,3$	$21,5 \pm 3,1$
SMM (kg)	$24,2 \pm 5,4$	$26,5 \pm 5,9$
Fat Mass (kg)	$16,1 \pm 5,9$	$13,3 \pm 5,1$
WHR (cm)	$0,86 \pm 0,04$	$0,83 \pm 0,04$
Waist line (cm)	$71,5 \pm 7,5$	$71,3 \pm 11,7$

Values are shown as means \pm Standard Deviation (SD)

In order to compare the two groups in terms of BMI, WHR, SMM, fat mass and waist line means, the Kaiser-Meyer-Olkin ($KMO = 0,65$) and Bartlett's Test of Sphericity ($p < 0,05$) were applied, and then a multivariate analysis of variance (MANOVA) was applied, but no significant differences were observed ($p > 0,05$).

In terms of clinical history of the subjects, some were taking other medication besides the birth control pill. The presence of family history of diabetes was also assessed. A Chi-Square test was applied to evaluate if there were significant differences in the prevalence of family history of diabetes between the two groups. Since some of the premises weren't validated, a Fisher's Exact Test was used to obtain the value of p ($p = 0,680$), but no significant differences were found in the prevalence of family history of diabetes between the two groups.

Table 6 – Clinical history of the subjects

	<i>Group A</i>	<i>Group B</i>
Medication		
None	7	8
Birth Control Pill	3	1
Others	2	3
Anti-inflammatory	2	
Antidepressant		1
Antiepileptic		1
Anti-arrhythmic		1
Family history of Diabetes		
Yes	6	8
No	6	4

Energy, macronutrient and dietary fiber intake on the day prior to the intervention were estimated and differences in the means of alimentary intake between the two groups were evaluated by Mann-Whitney U test, since the variables didn't have a normal distribution. However, no significant differences were found. The results are expressed in **table 7**:

Table 7 - Energy and macronutrient intake mean measurements in the day prior to the intervention

	<i>Group A</i>	<i>Group B</i>	<i>p</i>
Calories (kcal)	2076,1 ± 640,4	2273,2 ± 1283,8	0,729
Protein (g)	96,6 ± 30,0	88,2 ± 29,3	0,686
Carbohydrates (g)	263,9 ± 66,7	295,9 ± 202,3	0,564
Fat (g)	72,3 ± 39,4	76,6 ± 56,4	0,954
Dietary Fiber (g)	21,3 ± 9,8	22,9 ± 17,1	0,564

Values are shown as means ± SD

A multiple linear regression was applied to evaluate if the alimentary composition of the last meal on the day prior to the intervention (see **Table 8**) could influence the fasting glycemic (T0 moment) values of each group. After verifying the validation of the premises, no significant evidence was found ($p > 0,05$).

Table 8 - Energy and macronutrient intake mean measurements of the last meal in the day prior to the intervention

	<i>Group A</i>	<i>Group B</i>	<i>p</i>
Calories (kcal)	433,6 ± 288,7	430,4 ± 353,4	>0,05
Protein (g)	16,5 ± 16,9	22,2 ± 23,5	
Carbohydrates (g)	60,6 ± 39,3	41,8 ± 34,6	
Fat (g)	14,0 ± 12,1	17,4 ± 19,8	

Values are shown as means ± SD

After verifying the data normal distribution and homogeneity, an independent sample T-test was applied to average if there were significant differences in the mean time of overnight fast between the two groups. As observed in **Table 9**, despite the mean time of overnight fast in group B is slightly higher than the mean time of overnight fast in group A (10,79 h VS 10,46 h, respectively), no significant differences were found.

Table 9 – Mean time of overnight fast in group A and group B

	<i>Group A</i>	<i>Group B</i>	<i>p</i>
<i>Time of overnight fast (hours)</i>	10,46 ± 2,6*	10,79 ± 2,28*	0,744

*Values are shown as means ± SD;

4.3 Postprandial blood glucose response

To determine whether there were differences in postprandial blood glucose mean levels at different times (30, 60, 90 and 120 min), between the two groups, a repeated-measures ANOVA and mixed model was applied. The ingestion of the mousse with 3 g of *C. burmannii* did not result in a significantly lower blood glucose response at different postprandial times (30, 60, 90 and 120 min) than the reference meal did ($p > 0,05$).

For both groups, the maximum mean value corresponds to T30 (see **Table 10** and **Figure 7**).

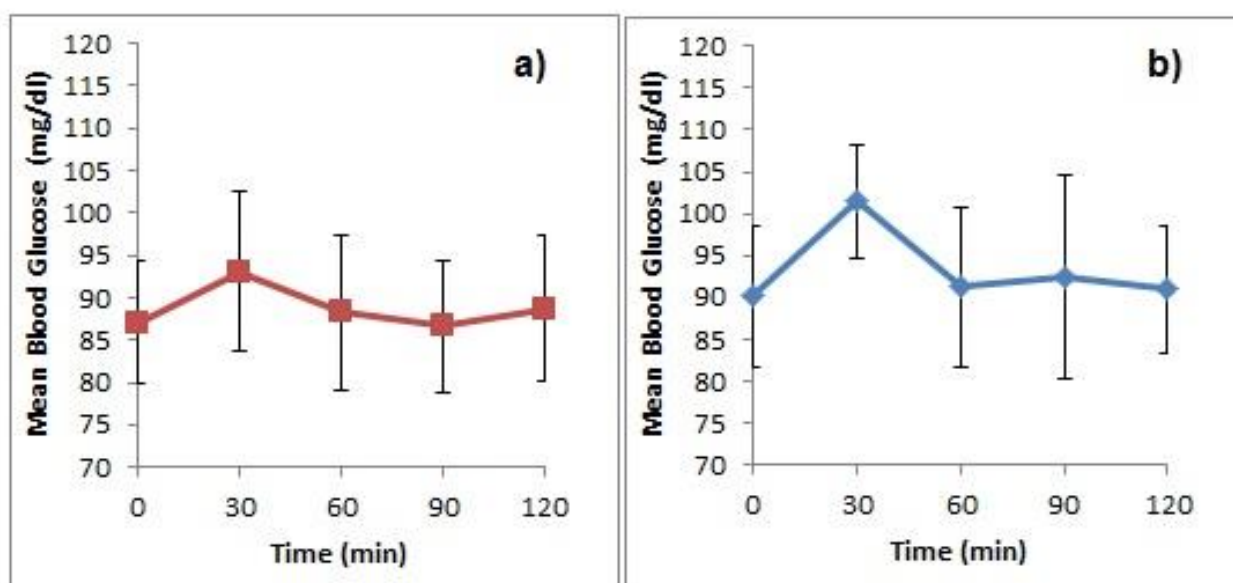


Figure 7 – Mean blood glucose concentrations (\pm SD) in healthy subjects after the ingestion of a mousse:
a) without cinnamon b) with 3 g of cinnamon.

Table 10 - Mean blood glucose concentrations in healthy subjects after ingestion of mousse with or without added cinnamon

	<i>Mousse without cinnamon</i>	<i>Mousse with cinnamon</i>	<i>p</i>
T0	87,08 ± 7,2	90,17 ± 8,5	
T30*	93,08 ± 9,3	101,42 ± 7,6	
T60	88,25 ± 9,1	91,25 ± 9,6	>0,05
T90	86,58 ± 7,8	92,50 ± 12,5	
T120	88,67 ± 8,6	91,00 ± 7,7	

Values are shown as means ± SD; *Maximum mean value

Significant differences in the mean values of Cmax, fasting blood glucose and AUC levels between the two groups were evaluated with the use of a T-test for independent samples.

As observed in **Table 11** and **Table 12**, the mean Cmax was significantly lower after the ingestion of the reference meal than after the ingestion of the mousse with 3 g of *C.burmannii* (96 mg/dl VS 104,42 mg/dl; p=0,011).

Table 11 - Individual blood glucose levels after the ingestion of a mousse without cinnamon (mg/dl), maximum concentration (Cmax) and respective means

<i>Subjects/Time (min)</i>	<i>0</i>	<i>30</i>	<i>60</i>	<i>90</i>	<i>120</i>	<i>Cmax</i>
1	74	95	86	88	84	95
2	89	91	76	90	81	91
3	89	92	89	77	92	92
4	92	101	104	97	96	104
5	81	92	94	76	95	95
6	92	110	92	98	99	110
7	81	86	73	80	82	86
8	83	75	77	80	74	83
9	99	90	92	98	102	102
10	90	85	86	86	92	92
11	95	105	93	84	86	105
12	80	95	97	85	81	97
Means (mg/dl)	87,08	93,08	88,25	86,58	88,66	96

Table 12 - Individual blood glucose levels after the ingestion of a mousse with 3 g of cinnamon (mg/dl), maximum concentration (Cmax) and respective means

<i>Subjects/Time (min)</i>	<i>0</i>	<i>30</i>	<i>60</i>	<i>90</i>	<i>120</i>	<i>Cmax</i>
13	88	113	86	84	87	113
14	91	91	99	108	90	108
15	92	99	89	98	94	99
16	76	101	77	70	80	101
17	95	96	102	87	98	102
18	76	97	89	80	91	97
19	94	93	82	96	82	96
20	95	115	85	89	92	115
21	82	109	106	103	90	109
22	90	97	83	83	82	97
23	101	103	92	99	101	103
24	102	103	105	113	105	113
Means (mg/dl)	90,17	101,41	91,25	92,5	91	104,42

It was also investigated whether there were significant differences in the mean values of fasting blood glucose between the two groups using an independent sample T-test, after verifying the data normal distribution and homogeneity. No differences were found ($p>0,05$).

The AUC of each subject is represented in **Table 13** as well as the respective mean values:

Table 13 – Postprandial blood glucose area under the curves (AUCs) in healthy subjects after the ingestion of a mousse without cinnamon or with 3 g of cinnamon.

<i>Subject</i>	<i>Mousse without cinnamon AUCs (mg.hr/l)</i>	<i>Subject</i>	<i>Mousse with cinnamon AUCs (mg.hr/l)</i>
1	10440	13	11115
2	10260	14	11655
3	10455	15	11370
4	11880	16	9780
5	10500	17	11445
6	11865	18	10485
7	9615	19	10770
8	9315	20	11475
9	11415	21	12120

10	10440	22	10470
11	11175	23	11850
12	10725	24	12735
Means	10673,75± 797,9*		11272,50± 808,2*

*Values are shown as means ± SD

No significant differences were observed in terms of the mean AUCs, of each group, for postprandial blood glucose responses ($p>0,05$).

To model the relationship between the BMI, fat mass and waist line with the AUC of the two groups, a multiple linear regression was applied. Taking into account the premises of this test and after removing the variable BMI of the model (Variance Inflation Factor >5), no association was found ($p>0,05$) with fat mass, waist line and the AUC values of the two groups.

4. Discussion

This study revealed differences in the phenolic composition and antioxidant capacity of the two meals. The chemical analysis showed that the mousse with 3 g of cinnamon has a much higher phenolic content than the mousse without cinnamon.

Type-A procyanidins are compounds present in cinnamon that have an antioxidant effect and also seem to exhibit insulin-like activity in cells (Anderson *et al*, 2004; Cao *et al*, 2007; Cao *et al*, 2010). Therefore, quantification of proanthocyanidins present in the mousse with and without cinnamon was measured. The quantification of proanthocyanidins revealed that the mousse with cinnamon has a higher amount of proanthocyanidins than the mousse without cinnamon. These results are consistent with the study of Shan *et al* (2007), in which high levels of proanthocyanidins were also identified in *C. burmannii*.

These results are consistent with those found with the antioxidant capacity tests, in which the mousse with cinnamon revealed a higher antioxidant capacity than the mousse without cinnamon.

In the FRAP assay, the values ranged from 91,1 to 941,4 $\mu\text{mol TROLOX/l}$ showing that the total antioxidant capacity of the mousse is ten times higher in the presence of cinnamon. The phenolic compounds present in cinnamon exhibit an antioxidant capacity, as observed by the range in the values of the FRAP assay, from 0,279 to 0,755 $\mu\text{mol TROLOX/mg phenols}$. Shan (2005), also found out that cinnamon stick contained very high levels of phenolic compounds (11.90 g of GAE/100 g of DW) and, in comparison with cinnamon cassia, this last one has a lower phenolic content (6,34 g of GAE/100 g of DW).

The ABTS \bullet + assay was used to estimate the total antioxidant capacity (TAS) and total equivalent antioxidant capacity (TEAC) of the two meals. In the tested conditions, *C.burmannii* showed a 50% inhibitory concentration achieved with 11,8 dilutions, while the mousse without cinnamon has no antioxidant capacity to reduce the ABTS radical at 50%. These results are in accordance with the ones observed by Murcia *et al* (2004) that compared the antioxidant properties of seven spices used in desserts with other antioxidant additives used in common food. When the TEAC assay was used to provide a ranking order of antioxidant activity, cinnamon was the spice with the highest total of antioxidant activity.

Despite the differences in the quantity of proanthocyanidins and the antioxidant capacity between the two meals, these differences don't seem to be enough to exhibit a lowering effect in the postprandial blood glucose values. This study showed that the ingestion of 3 g of cinnamon with a semi-solid food does not reduce the postprandial blood glucose response of healthy subjects. In fact, mean postprandial blood glucose levels tended to be higher after the ingestion of the test meal compared to the reference meal (see Table 8). Also, in terms of mean AUCs of each group, no significant differences were observed. These results are in accordance to a crossover study in nine healthy subjects (Markey *et al*, 2011), where the supplementation of 3 g of cinnamon simultaneously with a high-fat meal did not change the postprandial glycemic response. Another recent study in healthy subjects indicted that the addition of 3 g of cinnamon to a low-fat rice pudding test meal had no significant effect on gastric emptying (GE) rate neither in the blood glucose response in terms of the AUCs, between the different meals (Hlebowicz *et al*, 2009). As in this study, Hlebowicz *et al* (2009) also assessed if there were significant differences in the means Cmax between the two groups. Unlike Hlebowicz *et al* (2009), where no significant differences were observed between the mean values of Cmax, this study revealed the mean values of Cmax were significantly lower after the ingestion of the reference meal than after the ingestion of the mousse with 3 g of *C.burmanni*. These different results may be due to the fact that the sample in Hlebowicz *et al* (2009) study was a unique sample, in which the differences in postprandial blood glucose response to the ingestion of the different meals were analyzed in the same subjects, and *C. cassia* was used instead of *C. burmannii*.

When added to the same test meal, 6 g of cinnamon significantly delayed GE and reduced postprandial glycaemia, but the decrease in blood glucose concentration was more evident than the delay in GE rate, suggesting that GE cannot be the sole mechanism (Hlebowicz *et al*, 2007). Although the GE was not assessed in this study, there seems to be a relation between the amount of cinnamon consumed, the delay in gastric emptying and the reduction in postprandial blood glucose concentrations. Moreover, there are other factors that can influence the postprandial blood glucose levels, such as sample size, cinnamon specie and types of foods consumed.

Several clinical trials have been conducted on diabetic subjects. Unlike the clinical trials in healthy subjects, and although the amounts of cinnamon consumed are

variable, they all were long term studies (Khan *et al*, 2003; Safdar, *et al*, 2004; Mang *et al*, 2006; Soni, 2009). Most of them observed a reduction in fasting blood glucose, and Soni (2009) also observed a reduction in postprandial blood glucose levels. Therefore, the different results observed in the different clinical trials may not only be due to the amount of cinnamon consumed but also due to the type of sample and the time of the study itself.

The results of this study may not be fully comparable to the results of other ones, since the fasting blood glucose levels may also fluctuate due to other conditions, such as the high variability of the experimental conditions including the carbohydrate content of the last meal and the energy expenditure between the last meal and the measurement. Several food factors, such as the components in food, may also be responsible for the differences in glucose and insulin responses (Björck, Granfeldt, Liljeberg, Tovar & Asp, 1994). Fat, for example, slows down the digestive process and the co-ingestion of lipids and proteins has been demonstrated to reduce the glycemic response to carbohydrates by delaying gastric emptying and stimulating insulin secretion (Nuttall and Gannon, 1991; Björck *et al*, 1994; Moghaddam *et al*, 2006). Also, taking in to account the glycemic index (GI) of foods, this may vary between apparently similar foods. This variation may be due to differences in the physical and chemical characteristics of the foods but also to methodological factors. Similar foods may have different ingredients or may have been processed with a different method, resulting in significant differences in the rate of carbohydrate digestion and therefore in the GI value. (Foster-Powell, Holt & Brand-Miller, 2002). Thus, both the ingredients and method of process of the reference meal and test meal should be guaranteed to be similar, in order to avoid variations in the GI of the two meals.

In this study, the only difference between the reference meal and the test meal was that this last one had the addition of 3 g of cinnamon. Adding cinnamon to the mousse had very limited impact on its alimentary composition, being this change mainly due to the increase in the meal kilocalorie (kcal), which primarily comes from carbohydrates (mono and disaccharide sugars).

In addition to the factors of foodborne, there are individual factors that influence the glycemic response, such as the insulin sensitivity of each subject, the function of pancreatic β cells and daily variation of parameters metabolic (Brand-Miller *et al*. 2001). The control of these factors assumes greater importance in experimental studies,

and therefore, in the present study, the random allocation of participants in groups and the homogeneity between the groups can decrease the interference of individual factors in the results.

In terms of the anthropometric characteristics of the subjects, the homogeneity between the two groups was guaranteed, since there weren't significant differences in the mean BMI, WHR, SMM, fat mass and waist line among the two groups.

With regard to differences in the means of alimentary intake on the day prior to the intervention between the two groups, these were evaluated but no significant differences were found. Also, it was evaluate if the alimentary composition of the last meal on the day prior to the intervention could influence the fasting glycemic values of each group and no significant evidence was found. Plus, significant differences in the mean time of overnight fast between the two groups were also evaluated. Despite the mean time of overnight fast in group B is slightly higher than the mean time of overnight fast in group A, no significant differences were found, ensuring homogeneity between the groups.

Family history of diabetes is consider a non-modifiable risk factor for Type 2 Diabetes and therefore, subjects with a family history of diabetes may have alterations in the glycemic profile (Alberti, Zimmet, Shaw, 2007). Although more than half the subjects that participated in this study had family history of diabetes, no differences in their distribution between the groups were identified, which guarantees the homogeneity.

Thereby, the results obtained in this study, in terms of blood glucose levels, were not affected by the individual factors described above.

This study has some limitations, such as the fact that the sample technique was by convenience, being a small sample composed mainly by young, healthy subjects, not allowing these results to be generalize to other populations. However, the random allocation of participants in the groups and the homogeneity between the control group and the experimental group was guaranteed in order to decrease bias.

Also, the database used to analyze the 24 hour dietary recall answered by the subjects, isn't a Portuguese database, which could lead to some bias with regard to the alimentary composition of some foods and recipes. However, the inclusion of Portuguese recipes to the database reduces the bias.

Although the homogeneity between the groups was guaranteed and the dietary composition of the reference meal and the test meal was almost similar, the addition of 3 g of cinnamon to the mousse didn't reduce the postprandial blood glucose response of the subjects, but greatly increased the phenolic content of this meal.

These results emphasized the importance of phenolic compounds in the antioxidant behavior of cinnamon and also indicated that the phenolic compounds contributed significantly to the total antioxidant capacity of this spice.

5. Conclusion

The phenolic and antioxidant analysis revealed that the mousse with 3 g of cinnamon has a much higher phenolic content and total antioxidant capacity than the mousse without cinnamon. However, these differences in the antioxidant capacity of the two meals don't seem to be enough to exhibit a lowering effect in the postprandial blood glucose values. This study showed that the ingestion of 3 g of cinnamon with a semi-solid food does not reduce the postprandial blood glucose response of healthy subjects.

Although the dose of 3 g of cinnamon seems to exhibit a significant reduction in the mean fasting glucose levels in type 2 diabetics, higher doses of cinnamon are apparently required to have an influence in postprandial blood glucose of healthy subjects. Nevertheless, due to its high proanthocyanidins content, *C. burmannii* could be a better choice, when compared to other cinnamon species.

Hence, clinical controlled trials with higher doses of *C.burmannii* or a different sample, such as type 2 diabetics, are required to clarify the role of this specie in a semi-solid food in the postprandial blood glucose levels.

6. References

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ANNEX

ANNEX

Annex A – Ethics Committee of Instituto Superior de Ciências da Saúde Egas

Moniz authorization




Ex.ma Senhora
Catarina Amaral

Monte de Caparica, 15 de Janeiro de 2013

Ex.ma Senhora,

Venho comunicar-lhe que o Pedido de Parecer que submeteu à apreciação da Comissão de Ética da Egas Moniz, com o tema "Efeito da ingestão de uma mousse com canela *C. Burmannii* sobre os valores médios de glicémia capilar pós-prandial e seu poder antioxidante", foi aprovado por unanimidade.

Queira aceitar os melhores cumprimentos,

O Diretor do ISCSEM

Prof. Doutor Manuel Jorge de Queiroz Medeiros

c.c. – Prof^a. Doutora Alexandra Bernardo

Annex B – Informed Consent

Consentimento Informado

Monte da Caparica, 11 de Dezembro de 2012

Exmo. (a) Sr.(a),

No âmbito do Curso de Mestrado em Nutrição Clínica do Instituto Superior de Ciências da Saúde Egas Moniz, sob orientação da Professora Doutora Alexandra Bernardo, solicita-se autorização para a participação no estudo “Efeito da ingestão de uma mousse com canela *burmannii* sobre os valores médios de glicémia capilar pós-prandial e seu poder antioxidante” a estudantes do Instituto Superior de Ciências da Saúde Egas Moniz, com o objectivo de determinar se existe variação nos valores de glicémia capilar pós-prandial de indivíduos adultos, após a ingestão de uma mousse com canela versus a ingestão de uma mousse sem canela, assim como, caracterizar a capacidade antioxidante deste alimento com e sem canela.

A participação neste estudo é voluntária e implica:

- a) Preenchimento de questionário de história familiar de diabetes, de medicação que efetua, o sexo e a idade;
- b) Medição e pesagem em balança de bioimpedância para recolha de dados antropométricos;
- c) Medição do perímetro da cintura;
- d) 10 picadas no dedo para recolher uma gota de sangue (medição da glicémia capilar). Estas picadas serão efetuadas em momentos diferentes: 1 picada em jejum, antes da ingestão de uma mousse com canela, e as restantes 30, 60, 90 e 120 minutos depois do início da ingestão do alimento; este procedimento é repetido uma semana depois apenas com a alteração da mousse que não terá canela;
- e) Preenchimento de inquérito alimentar às 24 horas anteriores.

A informação recolhida nos questionários e os dados antropométricos recolhidos destinam-se unicamente a tratamento estatístico e publicação e será tratada pelo orientador e pelos seus mandatados. A sua recolha é anónima e confidencial (os dados dos participantes serão registados com recurso a uma codificação).

A sua não participação não lhe trará qualquer prejuízo.

Este estudo pode trazer benefícios ao progresso do conhecimento, uma vez que a canela *burmannii* é uma espécie ainda pouco estudada, sendo escassa a literatura publicada sobre o seu poder antioxidante nos alimentos, bem como sobre o seu papel nos valores de glicémia pós-prandial. A canela *burmannii* pode surgir, assim, como uma fonte de antioxidantes naturais, facilmente acessível. Como a canela não contribui para a ingestão calórica,

indivíduos saudáveis podem beneficiar da inclusão regular de canela *burmannii* na sua dieta, por forma a prevenir e/ou controlar níveis de glicémia elevados.

Este estudo pode trazer potenciais riscos a indivíduos que estejam a tomar fármacos metabolizados pelo citocromo P450, pois a canela pode interferir com as enzimas que metabolizam estes fármacos, segundo estudos *in vitro*. Quando utilizada em doentes que tomam agentes anticoagulantes, a canela pode aumentar o risco de hemorragia pois, com base em estudos animais, esta especiaria pode levar a uma diminuição do número de plaquetas no sangue.

A cumarina, presente em algumas espécies de canela, pode causar hepatotoxicidade a indivíduos com patologias hepáticas.

Está descrito que a canela apresenta propriedades antiarrítmicas bem como antilipídémicas, pelo que indivíduos que tomam agentes antiarrítmicos ou antilipémicos, devem evitar ingerir canela para que não haja interferência medicamentosa. Indivíduos que estejam a fazer algum destes tipos de medicação, não serão incluídos no estudo em questão.

Quando ingerida oralmente em doses diárias de até 6 gramas e até 6 semanas, a canela não apresenta riscos para uma população saudável e que não esteja a tomar nenhum dos fármacos acima descritos.

(riscar o que não interessa)

ACEITO/NÃO ACEITO participar neste estudo, confirmando que fui esclarecido sobre as condições do mesmo e que não tenho dúvidas.

X

Assinatura do participante

X

Assinatura do Orientador

Annex C – Sheet recorder of anthropometric data

Folha de Registo de Dados Antropométricos

Data: __/__/____

Nº de identificação: __

Peso	Altura	IMC	MME	MG	RCA	PC

Annex D - 24 hour dietary recall

Inquérito alimentar às 24 horas anteriores

Nº de identificação: ____

Data: ____/____/____

1. Tente lembrar-se do seu dia de ontem:

1.1 A que horas acordou? _____

1.2 A que horas adormeceu? _____

2. Tente descrever tudo o que comeu e bebeu durante o dia de ontem:

Hora	Local	Alimentos/Bebidas Quantidades/Tipos de confeção

3. O dia de ontem foi um dia alimentar normal? Sim____ Não____

3.1 Se não foi o que foi de diferente?

Nota: Alimentos ou bebidas com cafeína, Álcool, Tabaco...

Annex E – Sheet recorder of clinical data of the subjects**Folha de Registo de Dados dos participantes**

Data: __/__/____

Nº de identificação: __

Sexo: __

Idade: __

- Antecedentes Pessoais (DM, doenças gastrointestinais, alergias..):
- Antecedentes Familiares (DM, doenças gastrointestinais, alergias..):
- Fumador: Sim__ Não __ Fumou nas últimas 8 horas? Sim __ Não __
- Prática de Exercício Físico: Sim__ Não__
- Está actualmente a tomar ou tomou no último mês algum destes medicamentos:

Anticoagulantes __

Antilipidémicos __

Antiarrítmicos __

Antiinflamatórios __

Outro __ Qual? _____

Valores de Glicémia Capilar	Hora:	Hora do início da ingestão:	Hora:	Hora:	Hora:	Hora:
	Glicemia t0		Glicemia t30	Glicemia t60	Glicemia t90	Glicemia t120

*EFFECT OF THE INGESTION OF A MOUSSE WITH CINNAMON C. BURMANNII ON THE POSTPRANDIAL
BLOOD GLUCOSE RESPONSE OF HEALTHY SUBJECTS AND ITS ANTIOXIDANT CAPACITY*